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Antifeedant Effect, Biological Efficacy and High Affinity Binding of Imidacloprid to Acetylcholine Receptors in *Myzus persicae* and *Myzus nicotianae*

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Abstract: It is known from laboratory studies that tobacco-associated forms of *Myzus persicae* (Sulzer) and the closely related tobacco aphid *Myzus nicotianae* (Blackman) are often somewhat less susceptible to imidacloprid than non-tobacco strains of *M. persicae*. Choice tests (floating leaf technique) showed that tobacco aphids were also less susceptible to the antifeedant potential of imidacloprid in contact bioassays. Synergists like piperonyl butoxide or DEF did not enhance the susceptibility of tobacco-associated morphs of *Myzus* ssp. to imidacloprid, thus providing evidence that neither oxidative detoxication nor hydrolytic metabolization took place. However, in an attempt to study the influence of endosymbiotic bacteria on the efficacy of imidacloprid, we allowed small populations of tobacco aphids to feed on diets containing the antibiotic chlortetracycline prior to imidacloprid treatment. While the effectiveness of imidacloprid, i.e. lower LC_{50} values, could be improved in all strains, including the susceptible reference strain, there was no change in overall tolerance factors. In order to investigate any possible alteration of the target site, the affinity of imidacloprid and nicotine to nicotinic acetylcholine receptors in whole-aphid homogenates was measured. All strains (and clones) showed the same high-affinity binding sites and no detectable difference.

Studies using the FAO dip method revealed that the lower susceptibility of *M. nicotianae* is not restricted to chloronicotinyls like imidacloprid or acetamiprid, because other insecticides with different modes of action such as pymetrozine and fipronil were also affected in laboratory studies. It is considered that the observed tolerance to chloronicotinyls in certain strains of *Myzus* ssp. is a natural variation in response, probably not coupled with any known mechanism of resistance in this species complex. © 1998 SCI

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Key words: imidacloprid; *Myzus;* acetylcholine receptor; antifeedant; cartap

1 INTRODUCTION

Since its first description as a separate taxon, the tobacco aphid *Myzus nicotianae* (Blackman), which is closely related to the green peach aphid *Myzus persicae* (Sulzer), has been the object of several papers dealing with resistance of this species and the related tobacco-feeding morphs of *M. persicae* to different types of insecticides, including carbamates, organophosphates and pyrethroids.[1–5] During 1986–1988, numerous accounts of control failure against the tobacco aphid were reported in most tobacco-growing regions of the United States.[6] The predominant form of the normally

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green-coloured tobacco aphids became the red-coloured morph in most areas of the world. The red-coloured form has some advantages in surviving, developing and reproducing at temperatures above 25°C, thus probably explaining its predominance in the field.[7]

Surprisingly, some field populations or even laboratory clones of *M. nicotianae* showed reduced susceptibility towards the chloronicotinyl insecticide imidacloprid, although these aphids had never been in contact with imidacloprid before. The observed tolerance factors were in general very low and not obvious in the field at recommended application rates, but significant in specialized laboratory bioassays.[3,8] Furthermore, a positive cross-tolerance to nicotine has been described in these aphids, suggesting the possible selection of a mechanism to tolerate certain levels of nicotine which may also cover the chloronicotinyl insecticides.[3,5,8] Nicotine is one of the major alkaloids in tobacco plants, the natural host of *M. nicotianae*, but it is translocated in the xylem, while *Myzus* ssp. feed mainly in phloem tissue.[9] However, some authors suggested that one possible reason for the reduced susceptibility of *M. nicotianae* towards imidacloprid lies in the mutation of the target site, the nicotinic acetylcholine receptor.[8] This was found not to be the case for a tobacco-associated red-coloured morph of *M. persicae* from Japan which was closely related to *M. nicotianae*.[5] While it showed a 5–8 fold tolerance to imidacloprid, Nauen *et al.*[5] could demonstrate high-affinity binding sites for tritiated imidacloprid in homogenates of the Japanese strain as well as in a susceptible reference strain. There was no statistical difference in corresponding receptor binding curves.

The objective of the present work was to investigate the nature of imidacloprid tolerance in *M. nicotianae* using a range of biological, behavioural and biochemical assays, including the measurement of imidacloprid binding to nAChR in *M. nicotianae*.

2 MATERIALS AND METHODS

2.1 Insecticides and Chemicals

All insecticides used were of highest purity available. Imidacloprid was obtained in-house. Nicotine was purchased from Sigma (St. Louis, USA) and cartap was obtained from Promochem GmbH (Wesel, Germany). Fipronil (250 g litre^{-1} SC (Regent® 250SC), pymetrozine 250 g kg^{-1} WP (Chess® 25WP) and acetamiprid 200 g kg^{-1} WP (Mospilan® 20WP) were purchased from the manufacturers. Stock solutions of technical insecticides were prepared in acetone (10 g AI litre^{-1}) and diluted in aqueous solutions of 'Triton' X-100 (1 g litre^{-1}). Chlortetracycline was kindly supplied by Dr P. Meisner (Entomology, in-house). [^{3}H]Imidacloprid ($9{\cdot}25 \times 10^{14}$ Bq Mol^{-1}) was a gift from J. E. Casida (University of California, Berkeley). All other chemicals were of analytical grade.

2.2 Aphids

The susceptible strain NS of the peach-potato aphid, *M. persicae*, had been reared in the laboratory since 1967 under the conditions described below. Strain JR is a highly resistant red-coloured *M. persicae* from Japan, closely related to *M. nicotianae* as described previously.[5] 934E, a clone of a North Carolina (USA) population of *M. nicotianae*, was kindly provided by A. L. Devonshire (Rothamsted Experimental Station, Harpenden, UK). Another *M. nicotianae* strain (FR) collected from tobacco fields in France was kindly provided by Yves Bouchery (INRA, Colmar, France).[10] Distinction between *M. persicae* and *M. nicotianae* was based, in general, on the electrophoretic separation of glutamate oxalacetate transaminase allozymes.[11] All strains were reared on chinese cabbage at 22–23°C, 60% RH and 16 : 8 h L : D photoperiod.

2.3 Bioassays

The bioassay procedure used was a modification of the FAO-recommended aphid dip test.[12] After dipping of apterous adults in aqueous insecticidal solutions containing 'Triton' X-100 (1 g litre^{-1}), the aphids were transferred onto freshly excised cabbage leaves placed in plastic containers. The petioles of the cabbage leaves were inserted into small plastic tubes containing water. In this modified FAO-procedure, the percentage mortality of dipped aphids was evaluated 24 h and 48 h after dipping.[4]

The synergists piperonyl butoxide and DEF were dissolved in 'Triton' X-100 (1 g litre^{-1}) at a concentration of 10 mg litre^{-1}. The synergist at the concentrations chosen showed no effects on aphids when assessed after 48 h. The above-mentioned synergist solution was used to dilute a stock solution of 10 mg imidacloprid in 1 ml acetone. The starting test concentration for an aphid dip was 100 mg imidacloprid litre^{-1} in all cases. Percentage mortality was scored after 24 and 48 h.

The antibiotic chlortetracycline was administered orally to groups of aphids feeding on artificial double membranes (sachets) including an aqueous sucrose solution (150 g litre^{-1}).[13] The concentration of chlortetracycline in aqueous sucrose was 100 mg litre^{-1} and aphids were allowed to feed on sachets 24 h prior dipping with different concentrations of imidacloprid. Mortality was assessed after 24 and 48 h. All experiments were repeated at least three times with a minimum of five different concentrations. At each insecticide concentrations 10 to 30 aphids were dipped. Probit analysis was performed

using the POLO-computer program (LeOra Software, Berkeley USA).

2.4 Choice tests

The method used to elucidate the antifeedant action of different insecticides by contact and oral ingestion was modified according to Powell *et al.*[14] Leaf discs (25 mm diam.) were painted on their upper side with 40 μl of insecticidal solution in 'Triton' X-100 (1 g litre^{-1}). The concentrations chosen for painting were sublethal ($\leqslant LC_{10}$) as calculated from biotests performed with dipped leaf discs. Control leaf discs were treated with a solution of 'Triton' X-100 (1 g litre^{-1}) without insecticide. After drying, the painted leaf discs (insecticide-treated and control) were placed on the surface of water standing in a plastic Petri dish (85 mm diam.). Leaves were connected with a small plastic bridge, allowing the aphids to walk from one leaf disc to the other. The experiment was started after placing five adult seven-to-eight-day-old apterous aphids from synchronized populations onto each leaf disc. The set-up was covered with the lid of the Petri dish to catch the excreted honeydew droplets above each leaf disc. The distribution of the aphids as well as the number of honeydew droplets and immatures were evaluated after 24 h. The experiment itself was replicated at least 10 times for each insecticide and strain/clone of *Myzus* ssp. tested. Statistical analysis was performed with the aid of the computer program INSTAT (GraphPad Software, Inc.). Data were analyzed using the following tests: ANOVA, Kruskal-Wallis nonparametric ANOVA test, unpaired *t*-test and Mann-Whitney *U*-test.

2.5 Nicotinic acetylcholine receptor binding studies with [^{3}*H*]imidacloprid

Binding of imidacloprid to aphid nicotinic acetylcholine receptors was measured according to Liu and Casida[15] with some minor modifications. Whole aphids (1000 mg), stored frozen in liquid nitrogen before use, were homogenized in a blender in sucrose solution (320 mM; 20 ml). After centrifugation for 10 min at 1200*g*, the supernatant was filtered through five layers of cheesecloth and used directly for binding assays. The assay (total volume 1 ml) consisted of potassium phosphate buffer (0·1 M; 0·7 ml, pH 7·4), containing 1 g litre^{-1} bovine serum albumin (binding buffer; 1 g litre^{-1}), homogenate (0·25 ml) and [^{3}H]imidacloprid (0·05 ml; 333 Bq = 0·36 pmol) in water containing methanol (16 g litre^{-1}). Unlabeled imidacloprid was added in binding buffer containing up to 0·02 μl dimethylsulfoxide. After incubation for 60 min at 22°C, ice-cold binding buffer (3 ml) was added, followed immediately by filtration through pre-wetted Whatman GF/C glass fiber filters and raising with ice-cold binding buffer (2 × 3 ml). Bound radioactivity was determined by scintillation counting of the filters. All values were measured in duplicates. pI_{50} values ($-$lg M of the concentration of cold ligand displacing 50% of bound [^{3}H]-imidacloprid) were calculated using a four-parameter logistic curve-fitting program (GraFit, Erithacus Software Ltd). Protein was determined according to Bradford[16] and standard calibration curves to calculate protein content in aphid homogenates were established using bovine serum albumin.

3 RESULTS AND DISCUSSION

3.1 FAO dip test

Most data fitted well using the probit analysis regression lines. In general the tests were aborted after 48 h, even though compounds like pymetrozine showed a somewhat slower action and lower LC_{50} values after 72 h. Even after incubation periods of 72 h, however, tolerance factors were in most cases within the same range. *M. persicae* NS was the most susceptible strain with all compounds tested. Tables 1a and b summarize the results obtained using the aphid dip technique. All insecticides tested were highly effective, with LC_{50} values in the same range. Strain JR and clone 934E showed tolerance factors between 6 and 10 against imidacloprid. Tolerance factors to the open-chain analogue, acetamiprid, were more or less in the same range. Strain JR was somewhat more tolerant to acetamiprid than clone 934E. However, the computed 95% confidence intervals were overlapping, indicating that the difference is not significant (Table 1). The French field strain FR of *M. nicotianae* was nearly as susceptible as the reference strain NS. That strain losts its hardiness after rearing for several generations in the laboratory.[10] Apart from the chloronicotinyls, other insecticides with different modes of action such as pymetrozine and fipronil also showed repeatedly a slightly lower efficacy against strain JR and clone 934E (but differences between reference strain and the other strains were in most cases non-significant).

3.2 Efficacy of imidacloprid against *Myzus* spp. in conjunction with synergists

In order to investigate the possible involvement of oxidative degradation of imidacloprid, especially in strain JR and clone 934E, we tested the influence of synergists such as piperonyl butoxide on LC_{50} values of imidacloprid. Another synergist included was DEF, which is primarily active on hydrolytic enzymes, though hydrolytic cleavage of imidacloprid, due to its structure, is not likely. Figure 1 clearly indicates no effect of standard synergists on the efficacy of imidacloprid in any of the

TABLE 1

(a) Efficacy of Imidacloprid and Pymetrozine against *Myzus* ssp. in Aphid Dip Tests

Strain/Clone	*Imidacloprid* $LC_{50(48\ h)}$ (*mg litre*$^{-1}$)	*95% FL*[a]	*Slope*	*TF*[b]	*Pymetrozine* $LC_{50(48\ h)}$ (*mg litre*$^{-1}$)	*95% FL*	*Slope*	*TF*
NS (*M. persicae*)	0·47	0·37–0·59	1·99	–	0·66	0·31–1·1	1·36	–
JR (*M. persicae*)	3·4	2·5–4·5	1·35	7	3·4	0·91–15	0·73	5
FR (*M. nicotianae*)	1·0	0·42–2·4	0·64	2	not tested			
934E (*M. nicotianae*)	4·8	3·5–6·8	1·08	10	4·0	1·3–11	1·35	6

(b) Efficacy of Fipronil and Acetamiprid against *Myzus* ssp. in Aphid Dip Tests

Strain/Clone	*Fipronil* $LC_{50(48\ h)}$ (*mg litre*$^{-1}$)	*95% FL*[a]	*Slope*	*TF*[b]	*Acetamiprid* $LC_{50(48\ h)}$ (*mg litre*$^{-1}$)	*95% FL*	*Slope*	*TF*
NS (*M. persicae*)	0·53	0·13–2·2	1·08	–	1·03	0·73–1·4	1·88	–
JR (*M. persicae*)	2·3	0·66–8·9	0·94	4	16	8·3–29	2·63	16
FR (*M. nicotianae*)	2·8	0·88–10	0·85	5	not tested			
934E (*M. nicotianae*)	2·7	0·65–31	0·67	5	10	6·6–15	1·58	10

[a] 95% fiducial limits.

[b] Tolerance factor = LC_{50} other strains/clones/LC_{50} strain NS.

strains/clones investigated. Synergists showed no toxicity to aphids when administered without insecticide. Neither synergist was able to increase the susceptibility of strain JR and clone 934E, suggesting that imidacloprid tolerance is not due to piperonyl butoxide-sensitive oxidative metabolism or hydrolytic cleavage of imidacloprid or its aphicidal metabolites which might be produced.[17]

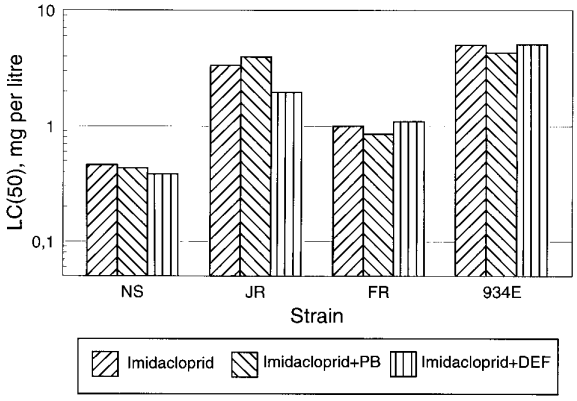

Fig. 1. Efficacy of synergists on imidacloprid toxicity in *Myzus* ssp. in aphid dip tests (48 h).

3.3 Potency of imidacloprid to *Myzus* ssp. after disruption of endosymbiotic bacteria

Another approach to elucidate the mechanism of tolerance to imidacloprid in strain JR and clone 934E was to assess the possible involvement of endosymbiotic bacteria, which may differ in different strains of *Myzus* ssp. The aphids had been maintained for 24 h on artificial diet containing chlortetracycline to become aposymbiotic. Afterwards the aphids were dipped in solutions of imidacloprid and scored for mortality after 48 h (Table 2). The susceptibility of chlortetracycline-treated aphids to imidacloprid was increased by factors of 10 and six in strains NS and clone 934E, respectively, thus indicating a possible influence of symbiotic bacteria on either hardiness or the efficacy of imidacloprid in both species. Further experiments are needed because we do

TABLE 2

Log-Dose Probit Mortality Data for Symbiotic and Aposymbiotic *Myzus* ssp. Tested with Imidacloprid

	Symbiotic				*Aposymbiotic*			
Strain/Clone	$LC_{50(48\ h)}$ (*mg litre*$^{-1}$)	*95% FL*[a]	*Slope*	*TF*[b]	$LC_{50(48\ h)}$ (*mg litre*$^{-1}$)	*95% FL*	*Slope*	*TF*
NS (*M. persicae*)	0·47	0·37–0·59	1·99	–	0·043	0·028–0·062	0·97	–
934E (*M. nicotianae*)	4·8	3·5–6·8	1·08	10	0·75	0·14–3·8	0·72	17

[a] 95% fiducial limits.

[b] Tolerance factor = LC_{50} strain NS/LC_{50} clone 934E.

not know if all symbiotic bacteria were affected after treatment with chlortetracycline for 24 h.

3.4 Binding of imidacloprid to nicotinic acetylcholine receptors in aphid homogenates

In order to examine whether the 10-fold tolerance towards imidacloprid of clone 934E in FAO dip tests was due to an altered receptor affinity, we investigated the receptor binding of imidacloprid in homogenates of susceptible *M. persicae* (strain NS) and the imidacloprid-tolerant clone 934E as a general representative for the sibling tobacco-associated species *M. nicotianae*. Furthermore, we measured the receptor affinity towards imidacloprid and nicotine in the Japanese *M. persicae* strain JR. These binding studies using aphid homogenates revealed no significant differences in displacement of [^{3}H]imidacloprid by unlabelled imidacloprid and nicotine between strains of *M. persicae* and *M. nicotianae*, suggesting that a receptor mutation which alters binding of imidacloprid and which may influence aphicidal potency is not the reason for imidacloprid tolerance in clone 934E. When measuring the displacement of radiolabelled imidacloprid by unlabelled imidacloprid and nicotine the resulting pI_{50} values for all populations of *Myzus* ssp. were 9·1 and 5·7, respectively (Figs 2a and b, Table 3). This is somewhat different from data presented previously.[5] Here all insect preparations may have been in a better condition and have contained more [^{3}H]-imidacloprid binding capacity (145 to 175 fmol [^{3}H]imidacloprid mg^{-1} protein versus 82 fmol [^{3}H]imidacloprid mg^{-1} protein) and less non-specific binding capacity (1·5 to 2·5 fmol mg^{-1} versus 10 fmol mg^{-1}). The observed differences in specific binding capacity between the three strains do not suggest differences in receptor density within the nervous system of the insect strain, since the capacities are related to the whole-body weights. In the present preparation there is only a very small and non-

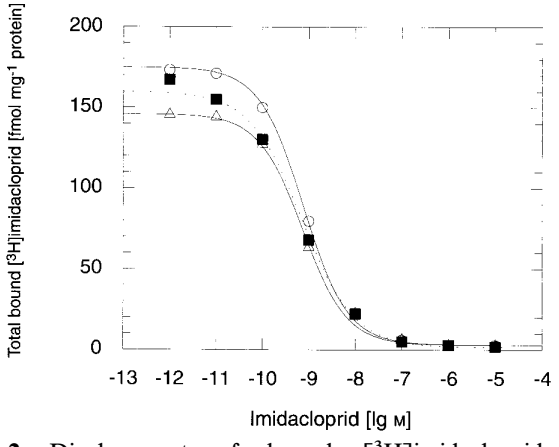

Fig. 2a. Displacement of bound [^{3}H]imidacloprid by unlabelled imidacloprid using homogenates of susceptible (NS, ○) and resistant (JR, ■; and 934E, △) strains of *Myzus* ssp.

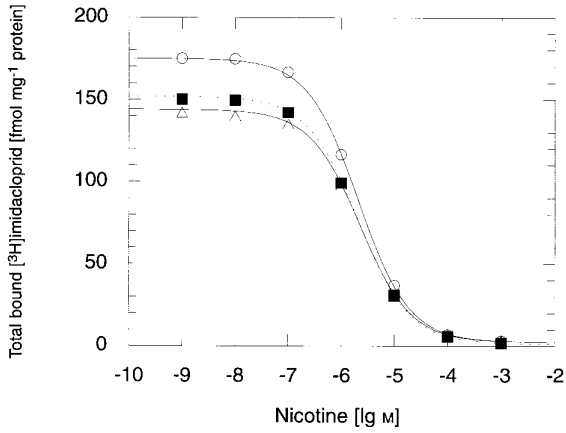

Fig. 2b. Displacement of bound [^{3}H]imidacloprid by nicotine using homogenates of susceptible (NS, ○) and resistant (JR, ■; and 934E, △) strains of *Myzus* ssp.

significant difference (max. 0·04) between the pI_{50} values of imidacloprid or nicotine in the different *Myzus* strains.

3.5 Effect of imidacloprid and cartap on behaviour of *Myzus* ssp.

When ingested orally, sub-lethal doses of imidacloprid have an antifeedant effect on *M. persicae*, with the result that affected individuals walk off treated leaves. If they do not then find an appropriate untreated leaf, they starve to death.[18] We performed choice tests with *M. persicae* and *M. nicotianae* using floating leaf discs to see whether there were any differences in the behavioural response of these species to foliarly applied sub-lethal concentrations of imidacloprid and cartap. Sub-lethal concentrations used were 0·1 mg litre^{-1} and 1 mg litre^{-1} for imidacloprid and cartap, respectively. Foliar-applied sub-lethal doses of imidacloprid had a distinct effect on the behaviour of *M. persicae* strains NS and JR, but with clone 934E of *M. nicotianae* the effects were much less clear. The distribution of adults of *M. persicae* strains NS and JR suggested an avoidance of the imidacloprid-treated leaf disc, because, after 24 h, significantly more individuals were found on the untreated leaf disc. The same occurred with the distribution of nymphs (Fig. 3). The number of honeydew

TABLE 3
Binding of Imidacloprid and Nicotine to nAChR in Homogenates of *Myzus* ssp. using [^{3}H]Imidacloprid as a Radioligand

	Imidacloprid		*Nicotine*	
Strain Clone	pI_{50}	*Hill coefficient*	pI_{50}	*Hill coefficient*
NS	9·12	1·1	5·68	1·1
JR	9·15	1·3	5·68	1·1
934E	9·13	1·1	5·64	1·1

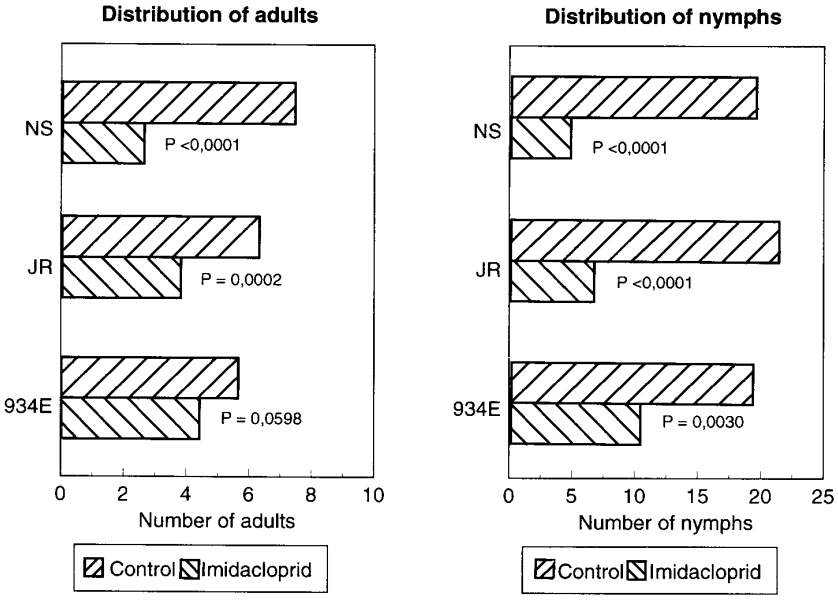

Fig. 3. Sub-lethal effects of imidacloprid (0·1 mg litre^{-1}) on *Myzus* ssp. feeding on foliar-treated and untreated leaf discs in choice tests (assessed after 24 h).

droplets excreted on the imidacloprid-treated leaf disc over the whole testing period was significantly lower than on untreated ones for both strains (Table 4). In the case of clone 934E, behavioural differences were less clear and, based on honeydew excretion at 24 h, both leaf discs were frequented equally. The distribution of nymphs after 24 h revealed that smaller individuals of clone 934E might be affected by sub-lethal doses of

TABLE 4
Average Number of Honeydew Droplets Excreted by *Myzus* ssp. on Imidacloprid-Treated and Untreated Control Leaf Discs

Strain	*Species*	*Imidacloprid 0·1 mg litre*$^{-1}$ (n *droplets*)	*Control* (n *droplets*)	*Significance Imi. vs. control*
NS	*M. persicae*	27 (±13)	111 (±18)	$P < 0{\cdot}001$
JR	*M. persicae*	46 (±10)	94 (±12)	$P < 0{\cdot}001$
934E	*M. nicotianae*	75 (±33)	83 (±30)	$P = 0{\cdot}435$

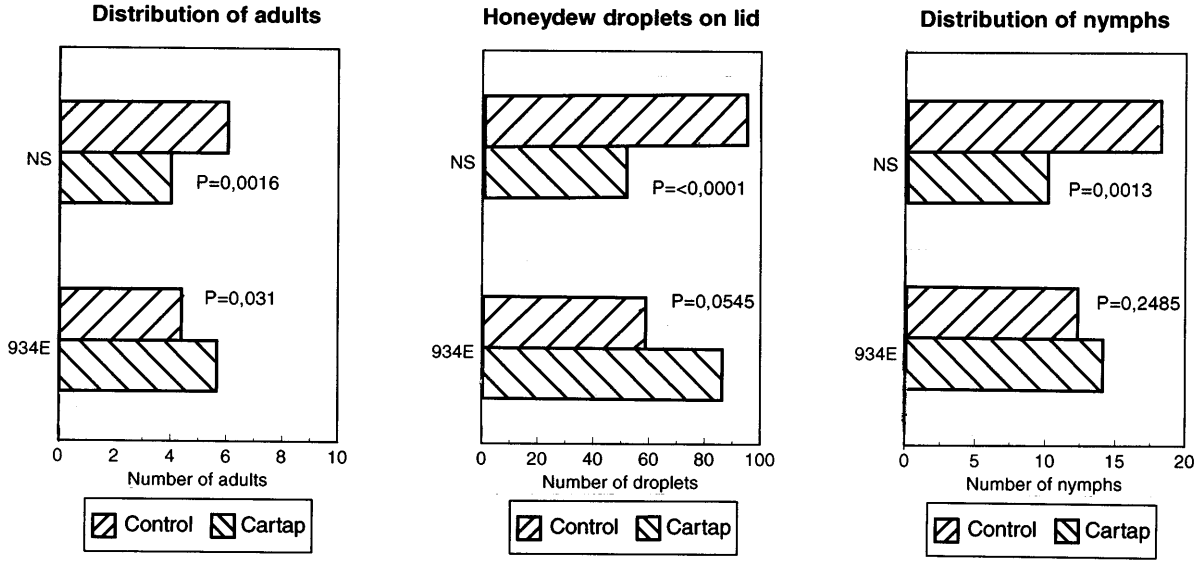

Fig. 4. Sub-lethal effects of cartap (1 mg litre^{-1}) on *Myzus* ssp. in choice tests using treated and untreated leaves (assessed after 24 h).

imidacloprid (Fig. 3). One-way analysis of variance (ANOVA) revealed that the average overall number of larvae and honeydew droplets within a single test (at least 10 replicates) was not significantly different for all strains of *Myzus* ssp. tested.

As shown with imidacloprid, foliar-applied sub-lethal doses of cartap altered the behaviour of *M. persicae* strain NS considerably. Cartap was much weaker in activity against aphids than imidacloprid, hence a concentration of 1 mg litre^{-1} was considered to be sub-lethal (LC_{10}). At this concentration no mortality was observed throughout the testing period of 24 h. The same observation was made with imidacloprid at 0·1 mg litre^{-1}. A significant preference for the untreated control leaf disc by *M. persicae* was found when counting the number of honeydew droplets excreted above each leaf disc. However, the distribution of adults and larvae of *M. persicae* also suggests an antifeeding potential for cartap (Fig. 4). Judged from honeydew excretion levels, *M. nicotianae* showed exactly the opposite preference, i.e. more honeydew droplets above the cartap-treated leaf disc, but statistically not quite significant ($P = 0{\cdot}0545$). However, when assessing the distribution of adults between cartap-treated and untreated leaf discs after 24 h, significantly more individuals were feeding on the treated leaf.

CONCLUSIONS

The results of the FAO dip test showed low but significant tolerance in clone 934E, a typical representative of *M. nicotianae* derived from North Carolina tobacco, to chloronicotinyl insecticides such as acetamiprid and imidacloprid. Compounds like pymetrozine and fipronil were also less active against clone 934E, but differences relative to the susceptible reference strain were non-significant, at least for fipronil. For fipronil, confidence intervals for all strains overlapped substantially and no conclusions can therefore be drawn regarding lower susceptibility in the imidacloprid-tolerant strains. However, baseline susceptibility of clone 934E compared to the reference strain NS of *M. persicae* was repeatedly lower for all compounds tested, suggesting a common natural hardiness rather than resistance based on biochemical mechanisms evolved to survive under insecticide pressure. Strain FR, a former field strain from tobacco in France, loses its tolerance to imidacloprid when reared under laboratory conditions, as shown very recently.[10] Imidacloprid tolerance in *M. nicotianae* or tobacco-associated *M. persicae* is widespread and correlates often with nicotine tolerance.[3,5,8] Clone 934E has never been treated with imidacloprid in the field, but was derived from tobacco and some authors have suggested that a possible reason for imidacloprid tolerance could be reduced receptor affinity, because of high levels of nicotine in tobacco plants.[8] However, nicotine is transported apoplastically, whereas the preferred feeding site of *Myzus* on tobacco is the phloem.[9] Nevertheless it is possible that small amounts of nicotine were imbibed on a regular basis when penetrating mesophyll cells in search of the phloem, as is suggested from analysis of electrical penetration graph recordings, where certain aphid species, including *M. persicae* also penetrate the xylem.[13,19] As shown for tobacco-associated morphs of *M. persicae*, *M. nicotianae* clone 934E showed no reduced affinity of the nicotinic acetylcholine receptor to imidacloprid and nicotine.[5] However, in fact, it could be clearly demonstrated for the first time that homogenates of *M. nicotianae* have the same high-affinity binding sites for tritiated imidacloprid as *M. persicae*. Hence target-site insensitivity can probably be excluded as a mechanism in low-level tolerance to imidacloprid of clone 934E and likewise in other strains of *M. nicotianae*.

Imidacloprid has strong antifeeding potential against *M. persicae* after oral ingestion in systemic biotests using excised leaves.[8,10,18] It is interesting to note that, in contact bioassays, the antifeedant effect of imidacloprid on *M. nicotianae* (clone 934E) is considerably lower than against *M. persicae*. However, it seems to be a common feature of *M. nicotianae* to tolerate sub-lethal doses of imidacloprid as shown in studies performed recently with a number of different clones.[8] The reason for this general tolerance remains unclear and it may be associated with the adaption of *M. nicotianae* to tobacco plants. In this connection, one of the most surprising results was the fact that, in choice tests, clone 934E showed an obvious preference for leaf discs treated with sub-lethal doses of cartap. The reason for this behaviour is not clear and it would be interesting to investigate further effectors of the nicotinic acetylcholine receptor to see if certain structural parts in a molecule determine whether a compound acting on the receptor as a neurotoxicant has also a potential as an antifeedant. It still remains unclear if this antifeedant property of imidacloprid is related to binding to acetylcholine receptors and therefore to a low-level intoxication, or to another still unidentified mechanism of action.

Piperonyl butoxide, an inhibitor of microsomal mixed function oxidases, did not enhance the effects of imidacloprid on clone 934E, i.e. co-application of this synergist with imidacloprid did not result in LC_{50} values closer to the reference strains NS, thus suggesting that there was no piperonyl-butoxide-suppressible oxidative potential in clone 934E to detoxify imidacloprid. The efficacy of piperonyl butoxide in *M. persicae* has been demonstrated in earlier studies, where it was found to synergize certain carbamates and the organophosphate parathion.[20,21] Another speculative hypothesis was the involvement of symbiotic bacteria which might be able to degrade imidacloprid to a

certain extent. In preliminary experiments we tested this hypothesis using the antibiotic chlortetracycline which has been successfully used to kill symbiotic bacteria in aphids.[22] The so-called aposymbiotic aphids of clone 934E became more susceptible to imidacloprid, but susceptible *M. persicae* of strain NS treated in the same way became even more susceptible, resulting in tolerance factors of >10.

In earlier studies it has been shown that the overexpressed esterases E4 or FE4 do not confer resistance to imidacloprid in *Myzus* ssp., hence we did not test the influence of esterases in this study.[3,5,8] Moreover, the observed factors of tolerance towards the compounds used throughout this study would never lead to a field failure at recommended application rates for *M. persicae* and *M. nicotianae*, respectively (Dobri, L., 1996, pers. comm.).

M. nicotianae or tobacco-associated *M. persicae* in general are aphid species, which often show a lowered susceptibility to chloronicotinyls (or *N*-heterocycle insecticides), probably due to their adaption to a plant rich in secondary plant metabolites such as alkaloids like nicotine. More detailed investigations in the future might reveal the mechanisms that are involved in the observed tolerance. Detoxication mechanisms like a putative alkaloid pump at its excretory organs/filter systems (malpighian tubules are lacking in aphids) have been reported for another nicotine-tolerant pest, the tobacco hornworm *Manduca sexta* (Joh.) but have not been described in aphids so far.[23]

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